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Enhancement of Focused Ultrasound Treatment by Acoustically Generated Microbubbles

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Microbubbles, whether introduced from outside the body or ultrasonically generated in situ, are known to significantly enhance the biological effects of ultrasound, including the mechanical, thermal, and sonochemical effects. Phase-change nanodroplets, which selectively accumulate in tumor tissue and whose phase changes to microbubbles can be induced by ultrasonic stimulation, have been proposed for high-intensity focused ultrasound (HIFU) tumor treatment with enhanced selectivity and efficiency. In this paper, a purely acoustic approach to generate microbubble clouds in the tissue to be treated is proposed. Short pulses of focused ultrasound with extremely high intensity, named trigger pulses, are used for exposure. They are immediately followed by focused ultrasound for heating with an intensity similar to or less than that of normal HIFU treatment. The localized generation of microbubble clouds by the trigger pulses is observed in a polyarylamide gel by a high-speed camera, and the effectiveness of the generated clouds in accelerating ultrasonically induced thermal coagulation is confirmed in excised chicken breast tissue. The use of second-harmonic superimposed waves as the trigger pulses is also proposed. The highly reproducible initiation of cavitation by waves with the negative peak pressure emphasized and the efficient expansion of the generated microbubble clouds by waves with the positive peak pressure emphasized are also observed by a high-speed camera in partially degassed water.

1. Introduction

1.1 Ultrasonic induction of irreversible biological changes

It is well known that radiation-based medical modalities, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and X-ray computed tomography (CT), use a level of energy much higher than that required to ionize molecules and break chemical bonds. Unlike radiation-based modalities, ultrasound in a uniform medium does not have a level of energy leading to such molecular changes. Figure 1 shows the order of magnitude of the molecular level of energy used in these medical modalities. The breakage of hydrogen bonds is considered to require the lowest molecular level of energy to induce irreversible changes to proteins, but the molecular level of ultrasonic energy is even lower than it by orders of magnitude. Therefore, ultrasonic energy needs to be accumulated or concentrated to induce irreversible changes to biological tissues.

Ultrasonic energy, similarly to many other forms of energy, can be accumulated as heat in the time domain. Microbubbles in fluidlike media can also concentrate ultrasonic energy in both the time and space domains. Figure 2 summarizes the mechanism of ultrasonically inducible biological effects, which can be used for therapeutic purposes. While ultrasound can induce thermal as well as mechanical effects by itself, microbubbles, whether introduced or ultrasonically generated, can significantly enhance these effects. Even chemical effects can be induced by the collapse of microbubbles.¹⁻⁸)

1.2 High-intensity focused ultrasound treatment

High-intensity focused ultrasound (HIFU) treatment, utilizing the thermal effect of ultrasound to coagulate the tissue to be treated, has been studied for more than half a century,⁹,¹⁰) and clinically used for more than a decade.¹¹⁻¹³) Figure 3 shows the numerically simulated rise in tissue temperature induced by a typical HIFU transducer for prostatic treatment. The transducer at 3 MHz had an aperture of 40×20 mm² and a focal distance of 30 mm. The heat conduction rate and blood perfusion rate of the tissue were assumed to be 5.5 mW cm⁻¹° C⁻¹ and 0.006 s⁻¹, respectively. The temperature rise was normalized by the maximum in each map. The energy deposition pattern induced by the focused ultrasound is close to the distribution of the temperature rise after 1 s exposure. The sharpness of the distribution is still maintained after 4 s exposure while it is lost upon 300 s exposure. Therefore, ultrasonic intensity high enough to
thermally coagulate the tissue at the focus within a relatively short time is used in HIFU treatment.

The sharpness of the distribution not only has the major advantage of providing the high geometric selectivity of HIFU treatment, but also has the disadvantage of the low throughput of the treatment. Figure 4 compares the numerically simulated temperature rise at the focal point and at an intervenient point in the case of 4 s exposure. The intervenient point is shown by a white + mark in Fig. 3.

At the intervenient point, although the temperature rise is smaller than that of the focal point by an order of magnitude, it is very slowly decreased after the exposure, almost only by perfusion, while it is decreased quickly by heat conduction at the focal point. Therefore, to protect intervening healthy tissues, a relatively long cooling time is needed between each consecutive HIFU exposure, which lengthens the overall treatment time in spite of the short coagulation time for each focal spot. Acceleration of the treatment is required to make HIFU treatment clinically more popular.

1.3 Acceleration of HIFU treatment by microbubbles

As shown in Fig. 2, the ultrasonic heating of biological tissues can be accelerated by microbubbles. Microbubbles, generated by HIFU itself, were reported to significantly enhance the thermal effect of HIFU.\textsuperscript{14–17} Microbubbles, administered to an animal in the form of encapsulated microbubbles, were also reported to accelerate the heating effect of HIFU by severalfold even at a dose within the range approved for ultrasonic diagnosis.\textsuperscript{18} However, if microbubbles are rather uniformly distributed in the body, they may significantly attenuate focused ultrasound before it reaches the focus. Therefore, microbubbles have to be somehow localized in the tissue to be treated for their utilization to accelerate HIFU treatment.

1.4 Phase-change nanodroplets

Microbubbles at the resonant size can convert ultrasonic energy at a high efficiency.\textsuperscript{17} Therefore, microbubbles with a diameter in the \(\mu\)m range are suitable to convert ultrasound in the MHz range, which is generally used for medical purposes because of the absorption roughly proportional to the frequency. However, this particle size is too large to obtain tumor tissue selectivity via the enhanced permeability and retention (EPR) effect. The volume as a liquid is smaller than the corresponding volume as a gas by three orders of magnitude. Therefore, if a microbubble can be delivered in the form of a droplet, the diameter of the droplet will be in the 100 nm range, which is known to be optimal for obtaining the EPR effect.

The concept of a phase-change nanodroplet is shown in Fig. 5. A mixture of two perfluorocarbons with boiling temperatures higher and lower than the body temperature is encapsulated and stabilized with phospholipids in the form of droplets with a diameter in the range of 100 nm.\textsuperscript{19–24} The phase change of the stabilized nanodroplets to microbubbles was induced by a short pulse of slightly focused ultrasound in the diagnostic intensity range.\textsuperscript{19} The high tumor selectivity of the perfluorocarbon droplets was observed by fluorine magnetic resonance imaging at 3 T\textsuperscript{20} and the acceleration of HIFU-induced thermal coagulation of tumor tissue was demonstrated, both in tumor-bearing animals.

Phase-change nanodroplets can be ideal for enhancing HIFU tumor treatment, not only in terms of efficiency but also in terms of selectivity, which is obtained as the synergistic product of their molecular selectivity and the geometric selectivity of HIFU. However, to clinically obtain this synergistic selectivity, approval will be needed for both...
phase-change nanodroplets and a HIFU instrument and also for their combination.

1.5 Acoustic generation of localized microbubbles

The acoustic generation of localized microbubbles was demonstrated using a short pulse of focused ultrasound with an extremely high intensity for histotripsy, in which the target soft tissue is emulsified by the mechanical effect of ultrasound enhanced by the microbubbles. The shortness of the ultrasonic pulse, whose length in space has the same order of magnitude as the focal depth of the field, is the key to making the well-localized generation of microbubbles possible.

Acoustically generated microbubbles can enhance not only the mechanical effects but also the thermal effects of ultrasound for therapeutic purposes. The enhancement of thermal HIFU treatment by acoustically generated microbubbles is demonstrated in this study.

Although the peak negative acoustic pressure is the most important factor for the acoustic generation of microbubbles or cavitation without depending on standing waves, its large magnitude is not easily achieved by ultrasound focusing because of the nonlinear propagation of high-intensity ultrasound followed by the focal phase shift. The peak negative focal acoustic pressure can be significantly smaller than the peak positive focal acoustic pressure by an order of magnitude in the pressure range typically above 10 MPa. However, the peak positive focal acoustic pressure was also reported to play an important role in the efficient formation of a cloud of microbubbles once they have been generated. The suggested mechanism behind this is that a cavitated microbubble acts as a pressure-releasing reflector that converts acoustic waves with highly positive peaks to those with highly negative peaks, which form a cloud of microbubbles in its vicinity.

The generation of acoustic cavitation was reported to be accelerated by superimposing the second harmonic on the fundamental for ultrasonic exposure. Either negative or positive peak pressure can be emphasized by controlling the second harmonic phase relative to the fundamental phase in this approach. Enhancement of the initial generation of microbubbles and the following formation of bubble clouds by waves with the negative and positive peak pressures emphasized, respectively, is also demonstrated in this study.

2. Materials and Methods

2.1 Ultrasonic exposure sequences

A typical sequence of focused ultrasound exposure in this study for HIFU treatment enhanced by acoustically generated microbubbles, named a triggered HIFU sequence, is schematically shown in Fig. 6. The initial ultrasonic pulses, named trigger pulses, used to generate microbubbles through acoustic cavitation, had an intensity higher than that for normal HIFU treatment by an order of magnitude. The following ultrasonic waves, named heating waves, had an intensity similar to or lower than those for normal HIFU treatment. The focal point of the trigger pulses may be quickly scanned when multiple clouds of microbubbles are formed. The scanning has to be fast enough to maintain the generated microbubble clouds. For the heating waves, their focal point may also be quickly scanned or a focal field with multiple foci may be used.

To test the efficiency of the acoustic generation of microbubbles by second-harmonic superimposition, waves with negative and positive peak pressure emphasized, as shown in Fig. 7, were used. Three different exposure sequences were tested: 1) exposure using the negative emphasized waves immediately followed by the positive emphasized waves, named the NP sequence, 2) exposure using the negative emphasized waves alone, and 3) exposure using the positive emphasized waves alone.

2.2 Ultrasonic sources

A 1–3 piezocomposite array transducer consisting of 128 elements aligned in the form of 4 tracks and 32 sectors at a central frequency of 1 MHz (Imasonic) was used to test the...
focused ultrasound at 1.14 and 2.28 MHz, respectively. The 28 elements painted in black were not driven. The input signals to the amplifiers were controlled so as to form a focal spot at the geometric focus as well as at the two points 4 mm laterally away from it for the heating wave exposure.30) An air-backed piezoceramic transducer consisting of two co-focally aligned spherically curved lead zirconate titanate (PZT) ceramic elements (Fuji Ceramics), as schematically shown in Fig. 9, was used to test the second-harmonic superimposed exposure. The outer and inner elements had resonance frequencies of 1.14 and 2.28 MHz, outer diameters of 78 and 34 mm, and spherical curvature radii of 80 and 72 mm, respectively. Each element was connected to one of the two outputs of an RF amplifier (E&I 100A2).32)

2.3 Calibration of ultrasonic amplitude and phase
The acoustic pressure from both transducers at the focal point was measured with a broadband hydrophone (Onda HGL-0085) in deionized, degassed water at relatively low intensities. Then, the ultrasonic intensity for each exposure was estimated assuming that it increases in proportion to the input electric power. The fundamental and second-harmonic phases from the dual-frequency transducer at the focal point were also measured at relatively low intensities, and tuned so that both negative and positive peaks were emphasized as shown in Fig. 7. Although a certain level of nonlinear distortion in the focal pressure waveform is expected, it was assumed that the phase relation does not significantly change as the ultrasonic intensity increases. This assumption was necessary to avoid destroying the hydrophone.

2.4 Ultrasonic exposure targets
To observe the microbubble clouds generated by the triggered HIFU exposure, a polyacrylamide (PAA) gel30,33) was used as the target. To test the thermal coagulation efficacy by the triggered HIFU treatment, blocks of excised chicken breast tissue were used as the targets. They were submerged in degassed water at 22°C with a dissolved oxygen (DO) level of 30–40%, which was achieved by degassing with a membrane degasser (ERC 3502W) at the maximum rate continuously in combination with a peristaltic pump. The geometrical focal point of the array transducer was located at a depth of 10 mm from the surface of the target for both cases.

To observe the effect of second-harmonic superimposed exposure on the generation of microbubble clouds, an anodic oxidized aluminum block was used as the target. It was submerged in degassed water at 24–25°C with a DO level of 70–80%. This DO level was achieved by degassing with the same degasser by adjusting the rate so as to maintain a reasonable level for the cavitation threshold of water. The geometrical focal point of the co-focal transducer was located at the surface of the target.

2.5 High-speed camera
To optically observe both microbubble clouds generated by either the triggered HIFU exposure or the second-harmonic superimposed exposure, a high-speed camera (Vision Research Phantom-V310) was used with an exposure time of 1 μs with back lighting. In the latter case, the back lighting was at an angle.

3. Results
Figure 10 shows the microbubble clouds generated in the PAA gel. Each trigger pulse was 100 μs long at a spatial-peak intensity of 32 kW/cm². Its focal position was scanned in the sequence of center, left, and right. This cycle was repeated 10 times and immediately followed by exposure to heating waves with triple foci at a spatial-peak intensity of 0.9 kW/cm² for 10 s. Figures 10(a)–10(c) show the inception of microbubble clouds at the central, left, and right focal points in the second, second, and fifth cycles, respectively. Figure 10(d) shows the microbubble clouds still maintained during the exposure to the heating waves. Figure 11 shows the gross crossovers of the blocks of excised chicken breast tissue after exposure to the heating wave at the same intensity and duration (a) with and (b) without exposure to the prior trigger pulse. The thermally coagulated tissue volume with the trigger pulses was a few times larger than that without them.30)

Fig. 8. Grouping of 4-track 32-sector piezocomposite array transducer elements to drive them by 10 outputs of RF amplifiers. The 28 elements painted in black were not driven.

Fig. 9. Schematic of dual-frequency co-focal PZT transducer for second-harmonic superimposed exposure. The outer and inner elements generate focused ultrasound at 1.14 and 2.28 MHz, respectively.
Figure 12 shows the effects of second-harmonic super-imposed sequences on the generation of microbubble clouds. Each exposure sequence at a spatial peak intensity of 3.5 kW/cm$^2$ was continued for 80μs in total. With the positive emphasized waves alone, no microbubble generation is observed. With the negative emphasized waves alone, a microbubble or small bubble cloud is observed to be generated, but it remains small. With the NP sequence, a microbubble or small bubble cloud, generated by the negative emphasized waves, is observed to expand to an extremely large cloud of bubbles by the following positive emphasized waves.$^{32}$ Here, it was estimated that the negative peak pressure of the negative emphasized waves was reduced by the nonlinear propagation by approximately 20% at this ultrasonic intensity level while the focal acoustic power was reduced by less than 5%.

4. Discussion and Conclusions

Figure 12 clearly demonstrates the advantage of the negative emphasized waves in creating microbubbles and that of the positive emphasized waves in growing the microbubbles to a large cloud of them. Cavitation microbubbles were created by the negative emphasized waves at a relatively low intensity, but the growth of the microbubbles to a cloud was suppressed during the exposure, which may be useful for certain purposes. The advantages of the proposed waves were demonstrated, although the second-harmonic phase

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**Fig. 10.** Microbubble clouds generated in PAA gel. The inception of microbubble clouds at the (a) central, (b) left, and (c) right focal points is shown. (d) Shows the microbubble clouds still maintained during the exposure to the heating waves.

**Fig. 11.** Gross crosssection of excised chicken breast tissue blocks after exposed to heating waves (a) with and (b) without prior exposure to trigger pulse.

**Fig. 12.** Effect of second-harmonic superimposed exposure sequences on generation of microbubble clouds. Each exposure sequence at a spatial peak intensity of 3.5 kW/cm$^2$ was continued for 80μs in total. The time elapsed after the start of exposure is labeled.
relative to the fundamental phase would have been tuned even better to emphasize either the negative or positive peak pressure if it had been based on the waveforms at the actual high acoustic pressure, which may be possible to measure using a fiber optic hydrophone.

An aluminum wall was used to ensure the reproducibility of the experiment. Its primary role was to prevent cavitated microbubbles from flowing away from the focal zone, and its secondary role was to double the ultrasonic intensity in the focal zone. Without the latter role, at least two times higher ultrasonic intensity would have been needed, which would have led to an even more significant effect of nonlinear propagation, and the tuning of the second-harmonic phase may have been very difficult. In the next step, the observed effects of the negative and positive emphasized waves should be confirmed in a medium closer to biological tissues such as a tissue-mimicking gel, in which the primary role above is not needed. If these effects are confirmed, the potential usefulness of this combined sequence of second-harmonic superimposed waves should also be considered for sonodynamic therapy\textsuperscript{1,2} and histotripsy.\textsuperscript{22–29}

Figures 10 and 11 also clearly demonstrate the significant acceleration of the ultrasonically induced thermal coagulation of tissue by the clouds of microbubbles generated by the extremely-high-intensity trigger pulses. As shown in Fig. 11(b), the tissue was coagulated only in the portion corresponding to the central focus of the triple foci of heating waves. This was probably because the heat dissipation away from the left focus to the left side and from the right focus to the right side was large enough to keep the portions of tissue below the coagulation temperature. The volume of coagulation with the trigger pulses, shown in Fig. 11(a), was slightly smaller in the propagation direction than that without the trigger pulses, shown in Fig. 11(b), probably because the clouds of microbubbles significantly attenuated the heating waves during their propagation. In the next step, the observed effects of the trigger pulses should be confirmed by in vivo experiments, where attenuation by the intervening tissues and cooling by the perfusion medium will have to be overcome. The latter may not be a significant problem because the thermal time constant of tissue is normally much longer than the duration of the heating waves.

The peak ultrasonic intensity of the trigger pulse used in this series of experiments is an order of magnitude higher than that of the heating waves, but it is still an order of magnitude lower than that of histotripsy pulses.\textsuperscript{22–29} Furthermore, the number of trigger pulses per treatment volume is two orders of magnitude less than that of the histotripsy pulses. Therefore, it is unlikely that the trigger pulses can induce such significant irreversible mechanical damage as histotripsy does.

Although this purely acoustic approach cannot use the molecular selectivity possessed by phase-change nanoparticles, it will only require approval for use with a HIFU instrument. If the negative and positive emphasized waves can be used as the trigger pulses for the controlled generation of microbubble clouds, the combined ultrasonic exposure sequence will be ideal for the acoustic generation of microbubbles to enhance HIFU treatment. This combined sequence is expected to have a triple synergistic selectivity, which arises from the synergy between the geometric selectivities of the negative emphasized waves to initiate cavitation, the positive emphasized waves to enlarge the generated microbubble clouds, and the heating waves to heat the microbubbles in the clouds. Because the cavitation threshold significantly varies in a body, imaging techniques to detect cavitated microbubbles will also be extremely important in making the proposed approach clinically useful.\textsuperscript{36}